

# Figure 1

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1006334.4E101

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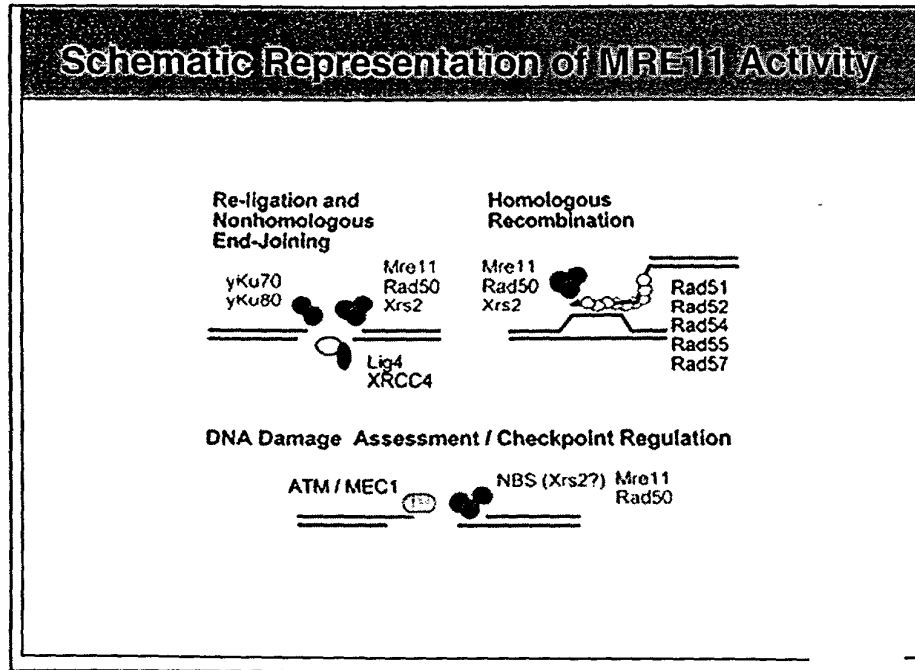
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**BOOK REVIEW**

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Figure 3



1002631.12901

Figure 4

## Dominant Negative Mutants Generated for Target Validation Studies

Two inactivating mutants were generated analogous to catalytically inactivating mutations in the yeast MRE11:

H217Y (MCB1998 Jan;18(1):260-68)

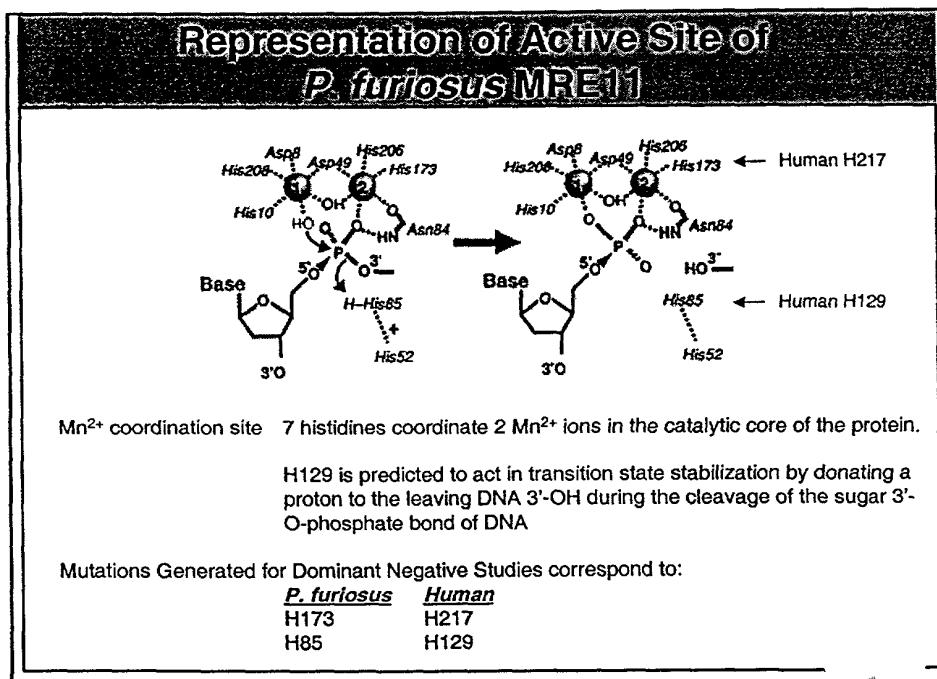
H129N (MCB1999 Jan;19(1):556-66)

Both histidines are thought to form part of the  $Mn^{2+}$  coordination site (7 histidines coordinate 2  $Mn^{2+}$  ions) in the catalytic core of the protein. H129 is predicted to act in transition state stabilization by donating a proton to the leaving DNA 3'-OH during the cleavage of the sugar 3'-O-phosphate bond of DNA

hMRE11	9	DENTFKILVATDIHLGFMKDAARGNDTFVTLDLRLAQENEVDIFLLGGDLFHENKPS	68
		D +T +IL+ TD R+G+ E D G+D++ T E++ LA+ N VD ++ GDLPH NKPS	
hMRE11	5	DPDTIRILITTDNHVGYNENDPITGDDSWKTFHEVMMLAKNNVDMVQSGDLFHVNKPS	64
	69	RKTLHTCLELLRKYCMGDRPVQFEILSDQSVNFGFSKFPWVNVQDGNLISIPVFSIHGN	128
		+K+L+ L+ LR CMGD+P + E+LSD S F + +F VNY+D N NISIPVF I GN	
	65	KKSLYQVLKTLRLCCMGDKPCELELLSDPSQVPHYDEFTNVNVEDPNFNISIPVFGISGN	124
	129	HDDPTGADALCALDILSCAGFVNHFGRSMSVEKIDISFVLLQKGSTKIALYGLGSIPIER	188
		HDD +G LC +DIL G +NHFG+ + +KI + P+L QKGSTK+ALYGL ++ DER	
	125	HDDASGDSLLCPMDILHATGLINHFQGVIESDKIKVPLLFQKGSTKIALYGLAAVRDER	184
	189	LYRMFVNKKVTMLRPKEDENSWFNLFIHQNRSKHGSTNFIPEQFLDDFIDLVIWNGHEHE	248
		L+R F + VT P E WFNL +HQN + H +T F+PEQFL DF+D+VIWNGHEHE	
	185	LFRTFKDGGVTFEVPTRREGWFNLMCVBQHTGHTNTAPLPEQFLPDPLDVIWNGHEHE	244
	249	CKIAPTKEQQLFYISQPGSSVVTSLSPGEAVKKHVGLLRIK-GRKMMHKKIPLHTVRQF	307
		C N + F + QPGSSV TSL EA K+V +L IK G M IPL T+R F	
	245	CIPNLVHNPIKNFVLPQGSVATSLCEAEAPKYVFILDIKYGEAPKMTPIPLETIRTF	304

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Figure 5



Summary of Target Validation Studies: MRE11							
Dominant negative studies							
Tumor A549	Antiproliferative Activity				Normal HMEC	HUVEC	PrEC
	Hela	PC3	H1299				
Wt							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
H217Y							
GFP-fusion	-	-	-	-	-	-	-
IRES GFP	-	-	nd	nd	-	-	nd
H129N							
GFP-fusion	++	++	-/+	-/+	-	-	-
IRES GFP	+	-	nd	nd	-	-	nd
Antisense: A549							inconclusive
( + indicates antiproliferative effect in either the GFP positivity study, cell tracker or antisense studies)							

(+ indicates antiproliferative effect in either the GFP positivity study, cell tracker or antisense studies)

Figure 7

Summary of Target Validation Studies: MRE11			
Dominant negative studies			
	Tumor A549	Chemosensitization Activity	
		Hela	HMEC
Wt GFP-fusion	-	-	-
H217Y GFP-fusion	++	++	-

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T02227.129201

Figure 8

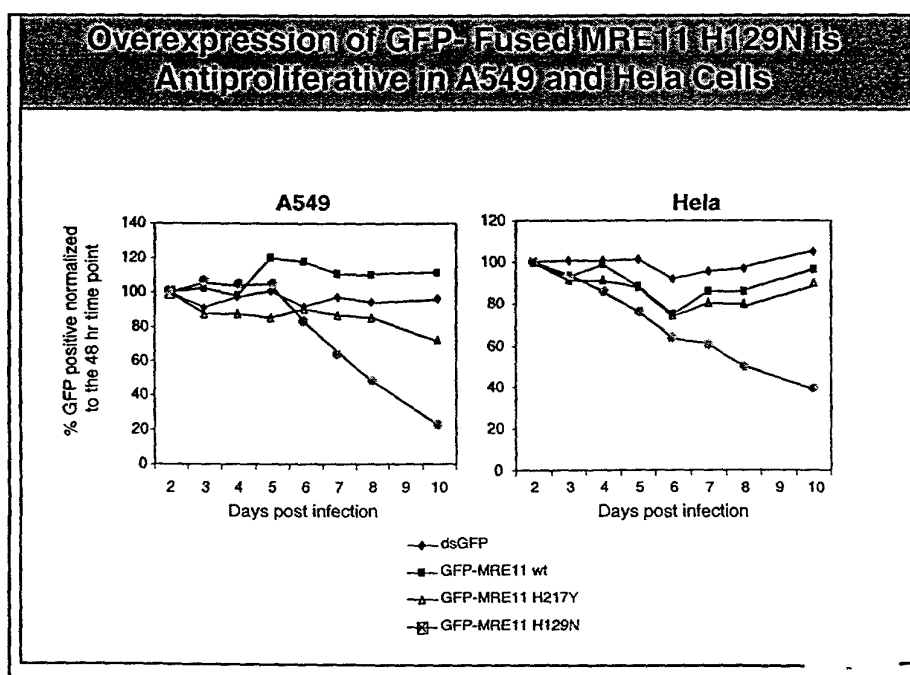




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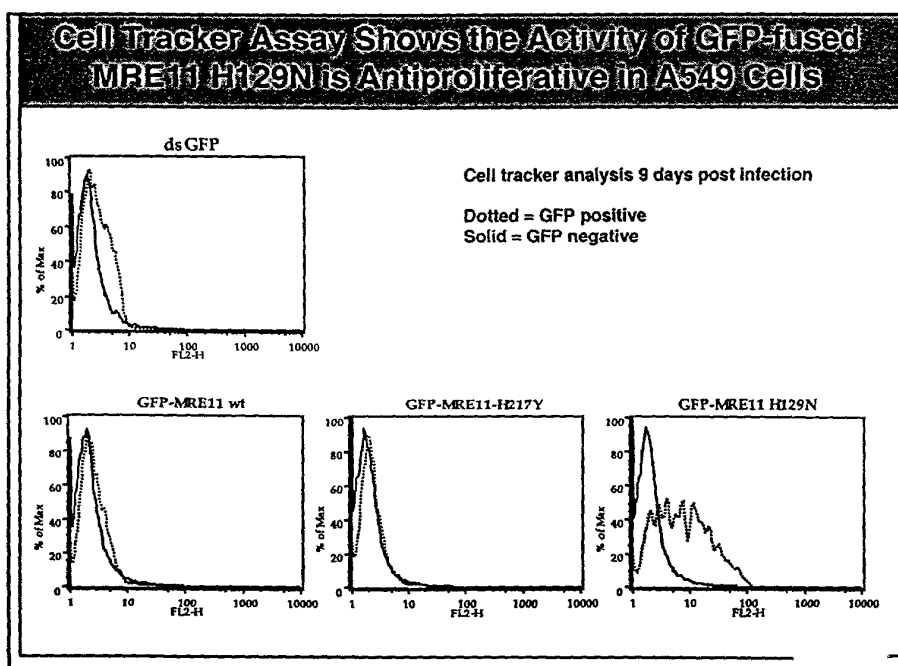
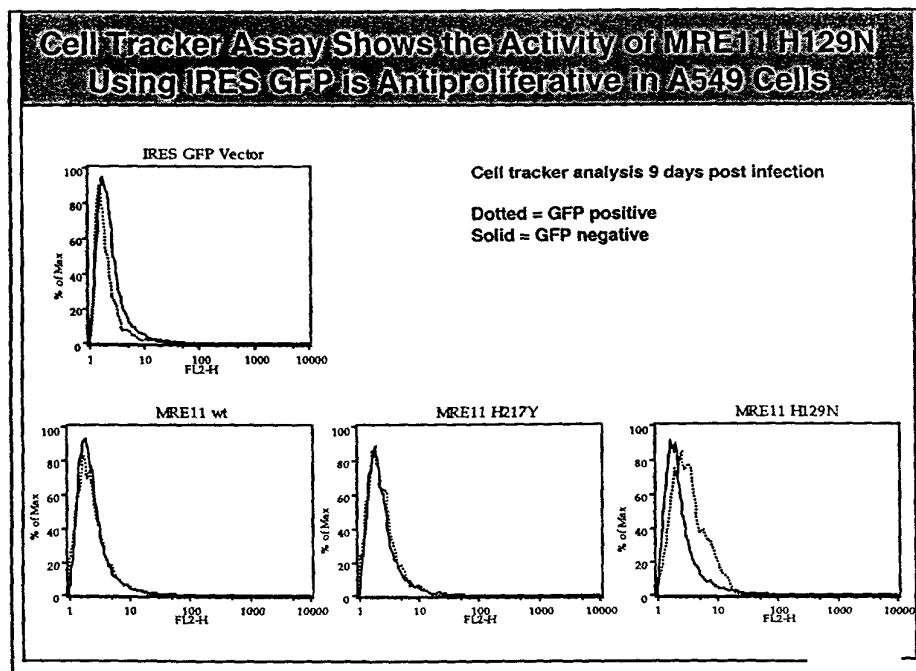


Figure 10



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Figure 11

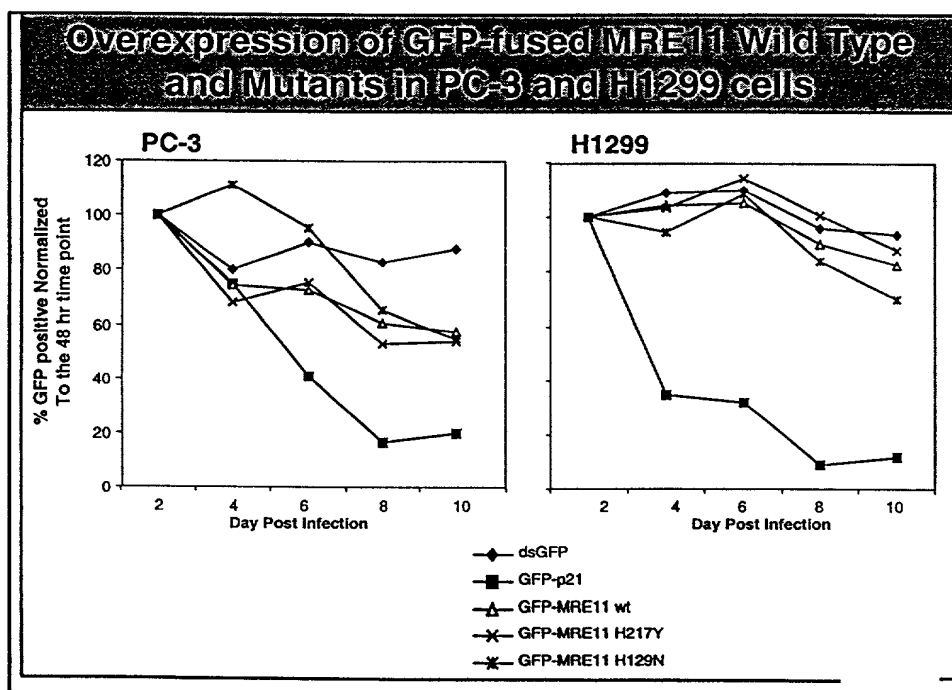


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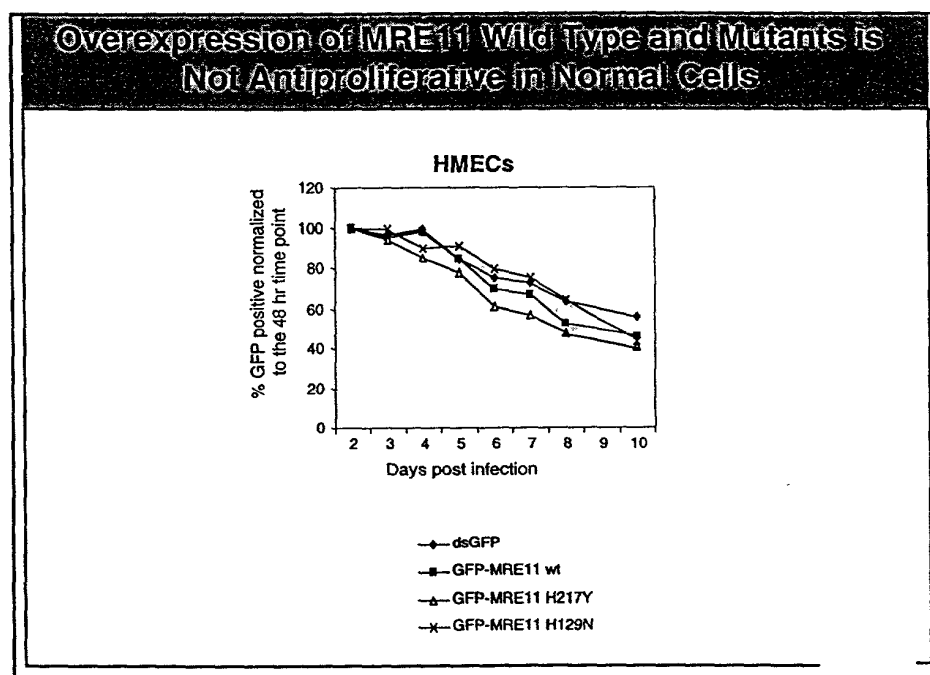
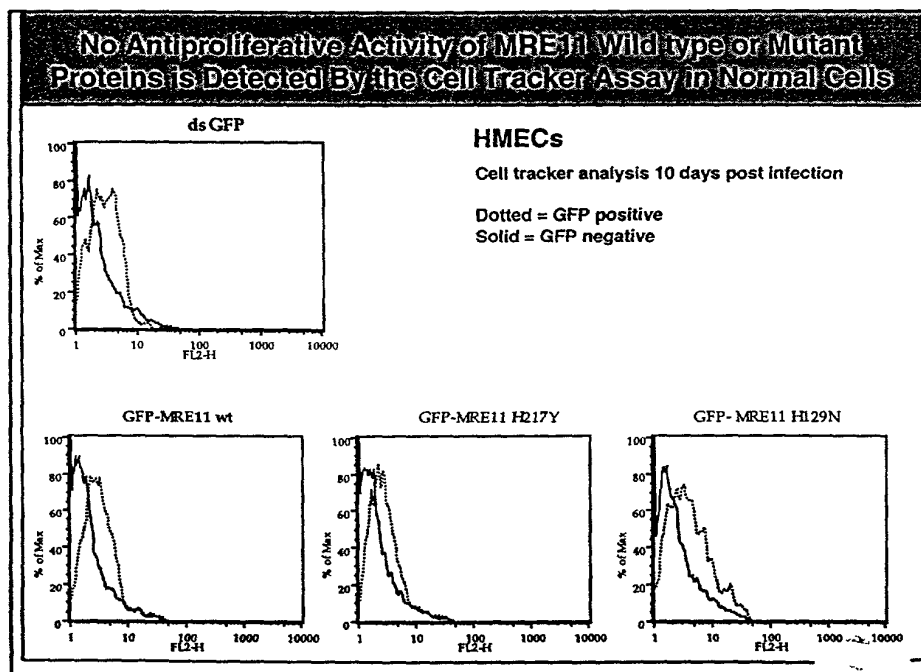


Figure 13



1003634-133404

Figure 14

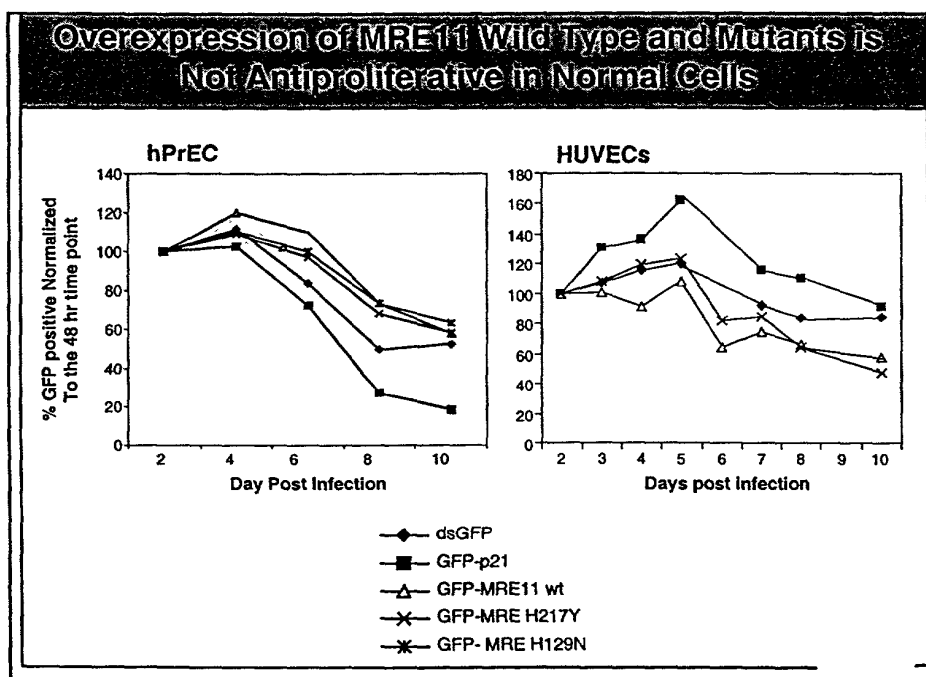


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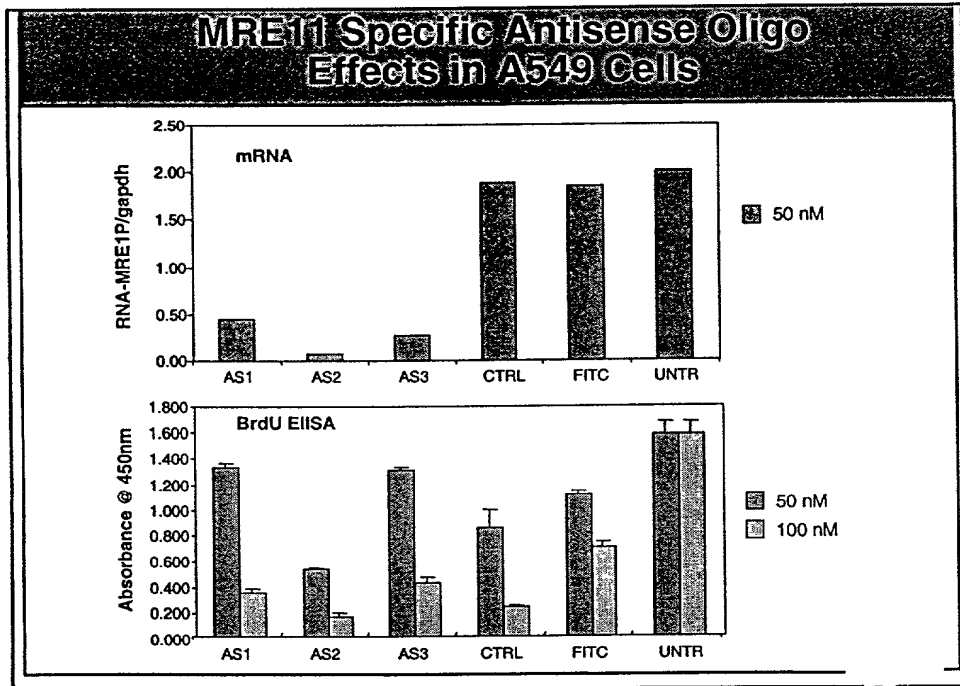


Figure 16

### Strategies for Assessing Chemosensitization Using Dominant Negative Studies

#### Plate based BrdU incorporation ELISA

Hela cells were infected with GFP-fused wt or mutant MRE11

The top 10% GFP positive cells were sorted 5 days after infection

Purified cell populations were plated in 96-well plates for  
chemotherapeutic treatments

BrdU incorporation was measured 48 and 72 after treatment

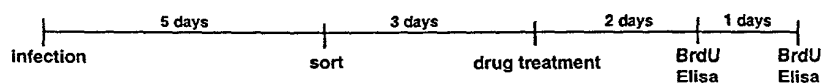




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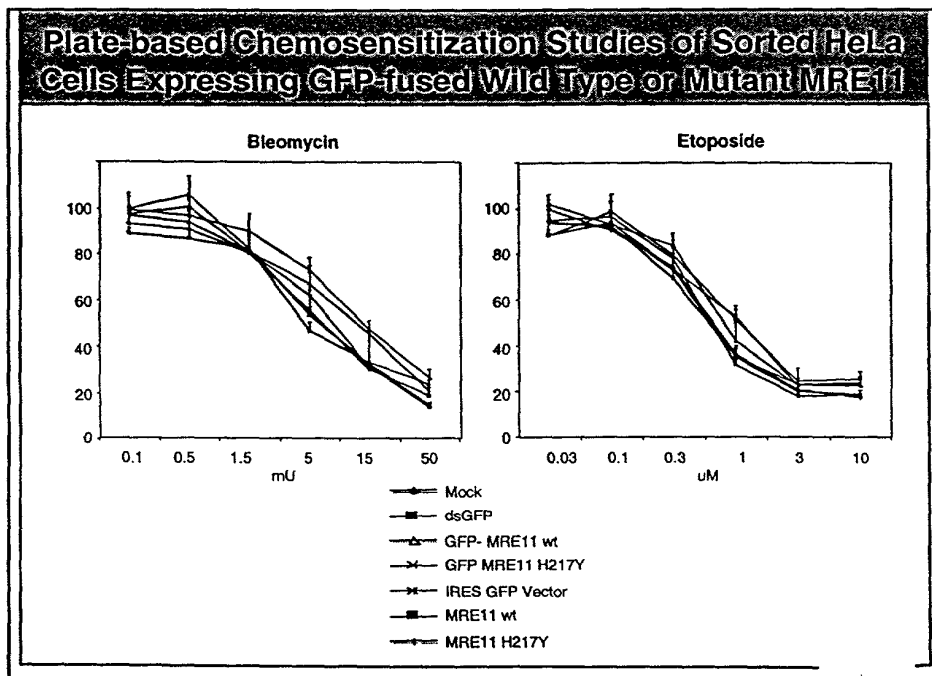


Figure 18

### Alternatives Strategy for Assessing Dominant Negative Chemosensitization Effects

Cell Survival after Drug treatment and wash out (similar to colony survival assay)

GFP positivity study to determine selective sensitivity of the GFP positive cells (reflecting cells expressing wild type or mutant proteins) in a mixed population of infected and non-infected cells

- Cells are infected with GFP-fused wt or mutant MRE11
- Cells are then treated with chemotherapeutics for 48 hrs
- After a 48 hr treatment period, the chemotherapeutic is washed out and cells are allowed to recover for 5-8 day.
- After the end of the recovery period, the %GFP positive cells in the treated population relative to the untreated population is assessed by FACS analysis

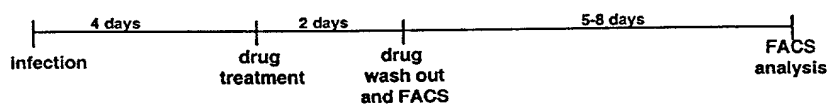
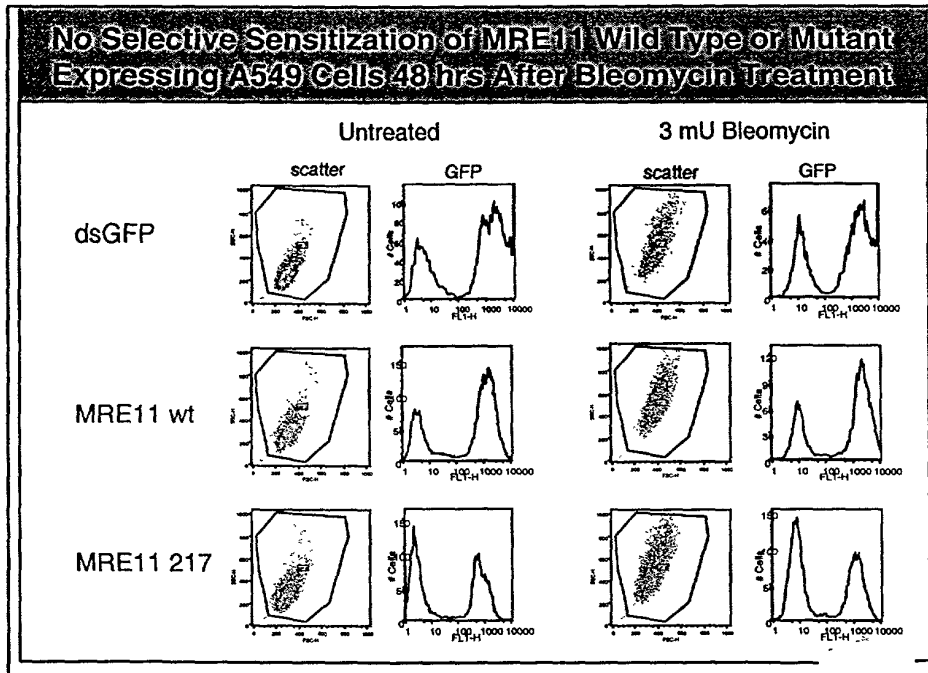


Figure 19



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Figure 20

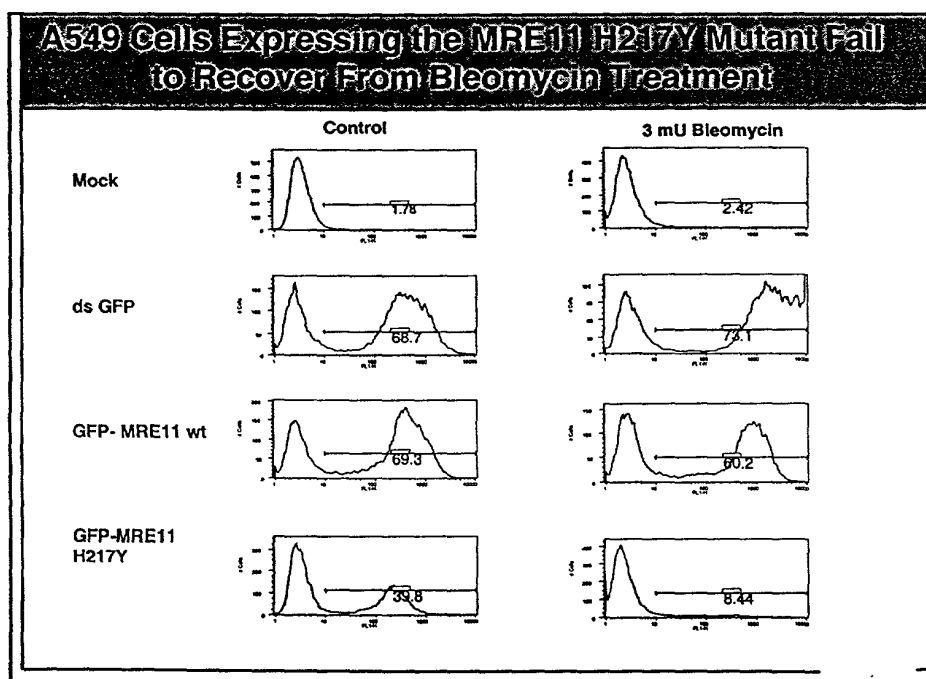


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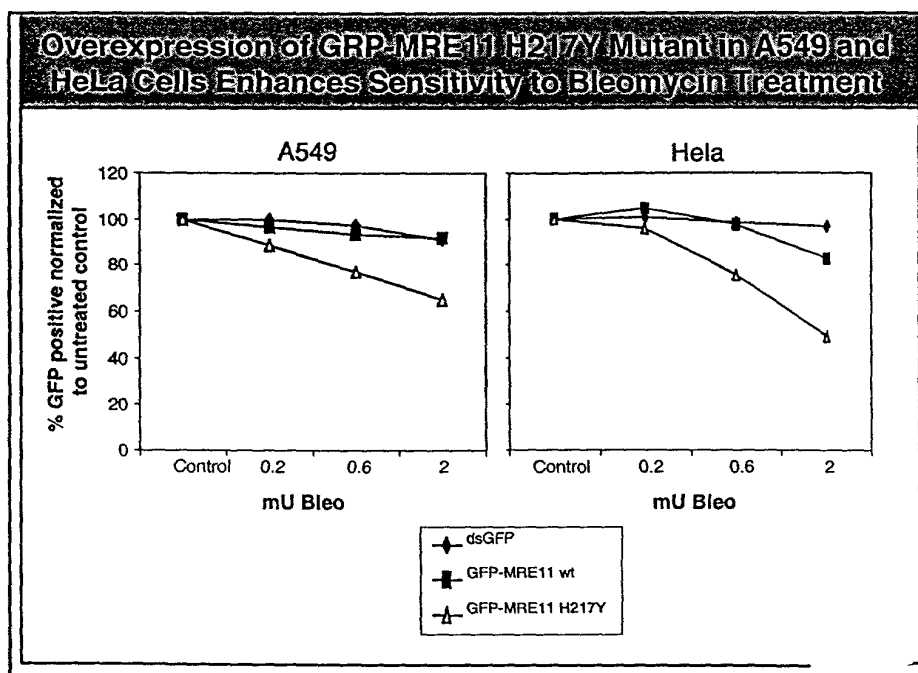


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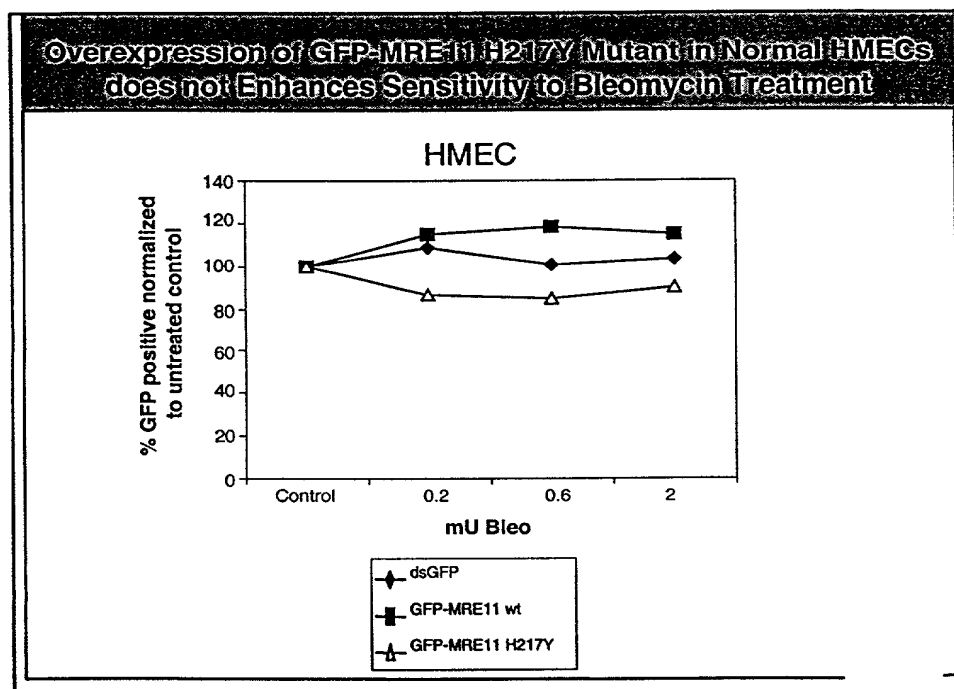
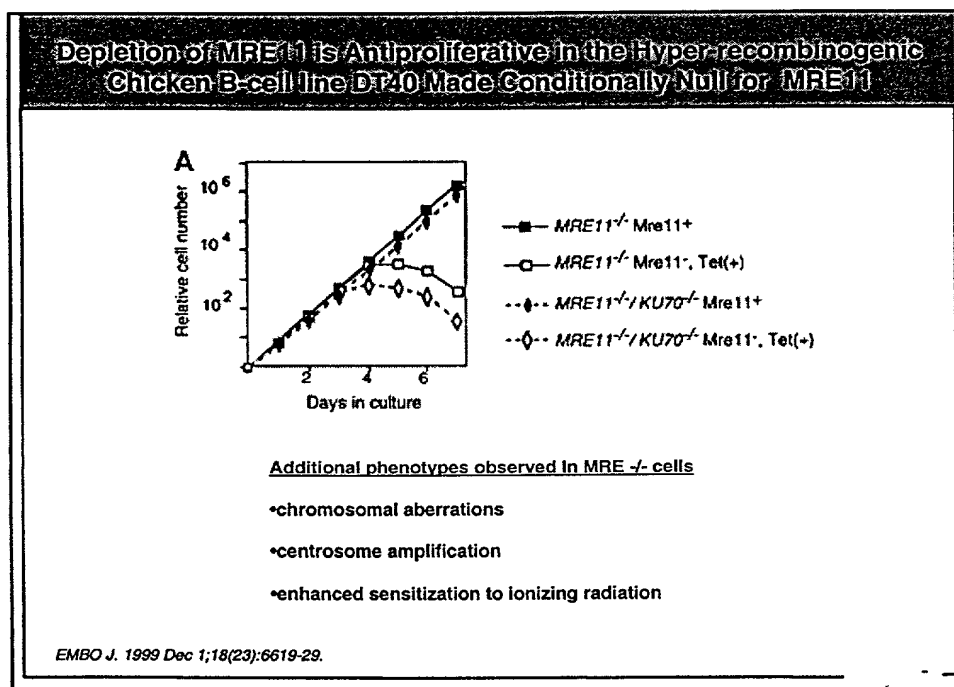


Figure 23



[illegible]

**Possible Models Explaining the Antiproliferative and Chemosensitization Effects of MRE11 Inhibition**

**Antiproliferative activity may be explained through MRE11's Role in:**

- Double strand break repair**
- Telomeric regulation**

### Antiproliferative activity may be explained through MRE11's Role in:

## Double strand break repair

## Telomeric regulation



Figure 25

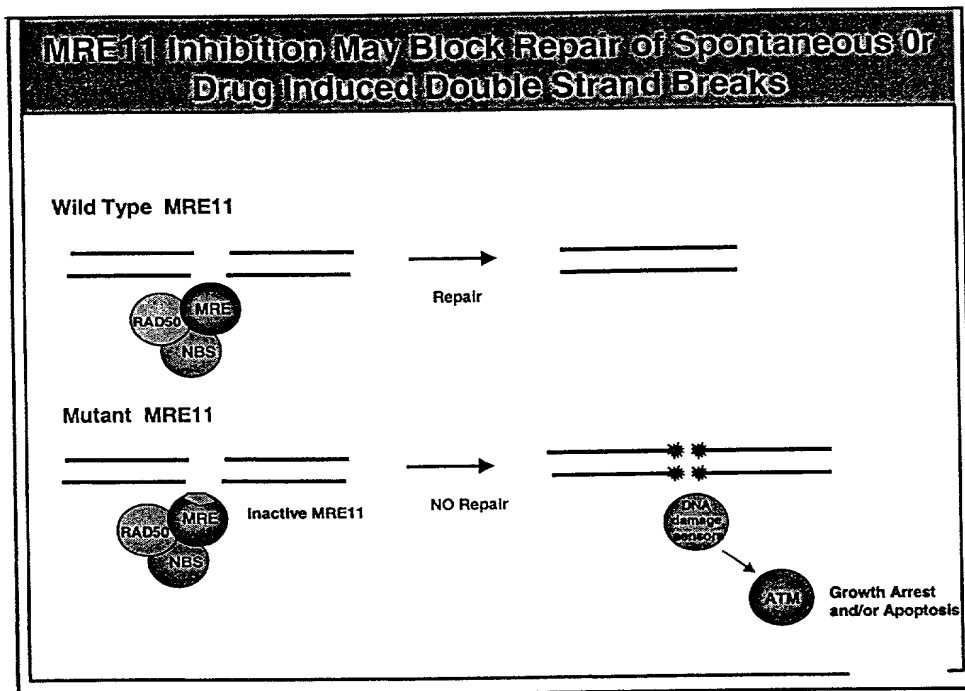


Figure 26

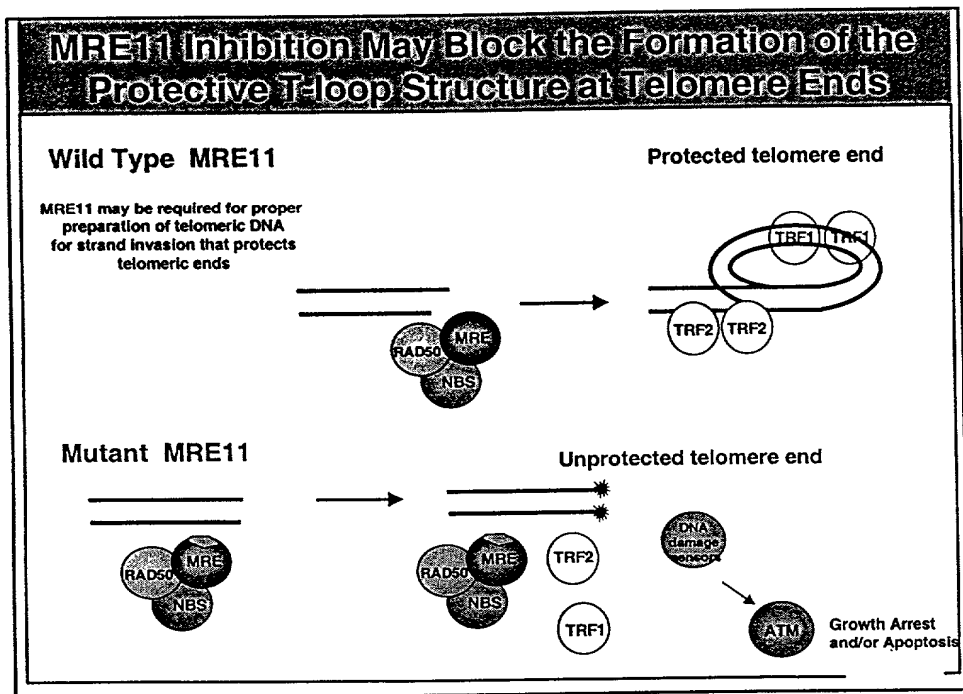


Figure 27

## MRE11 Summary

### Functional Studies

Source: YTH- PCNA/Nbs1

#### Antiproliferative Activity

- Overexpression of MRE11 H129N mutant protein is antiproliferative in tumor cells, but not in normal cells
- No strong antiproliferative effect is seen in cells expressing MRE11 wild type or H217Y mutant

#### Chemosensitization

- Overexpression of MRE11 H217Y mutant enhances sensitivity to chemotherapeutic treatment in tumor cells
- Sensitization by the H129N mutant cannot be assessed because of the inherent antiproliferative activity seen with expression of this mutant

#### Literature

- Numerous studies have suggested that MRE11 plays an important role in DNA damage repair pathway
- Studies on the yeast protein suggest that inhibition of catalytic activity of MRE11 will result in sensitivity to ionizing radiation

#### Conclusion

- Functional studies suggest inhibition of MRE11 will selectively inhibit tumor cell growth and enhance the response of tumor cells to DNA damaging agents

For "Footnote"

Figure 28

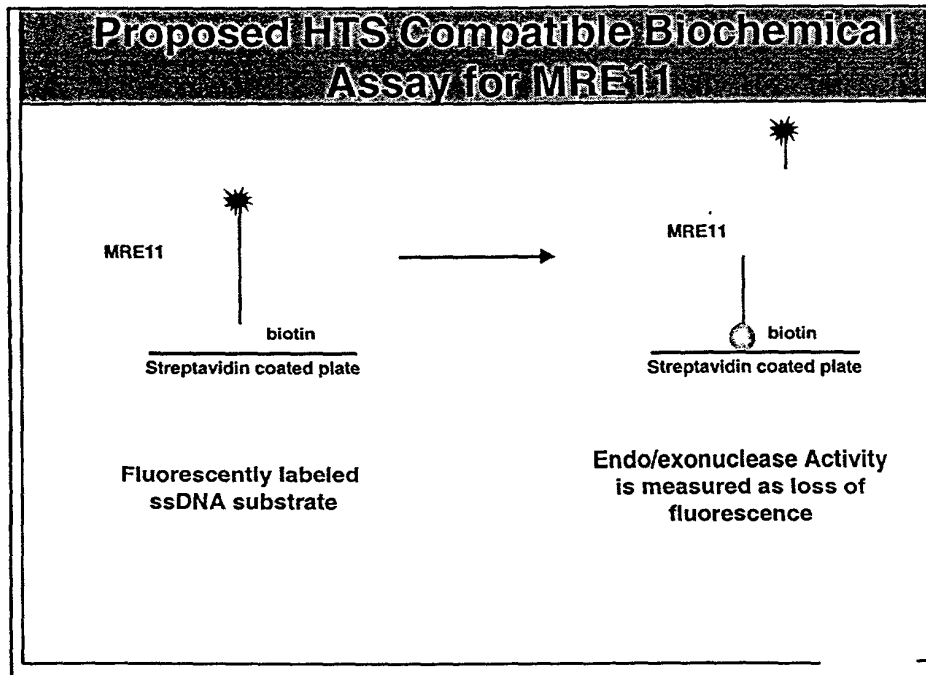
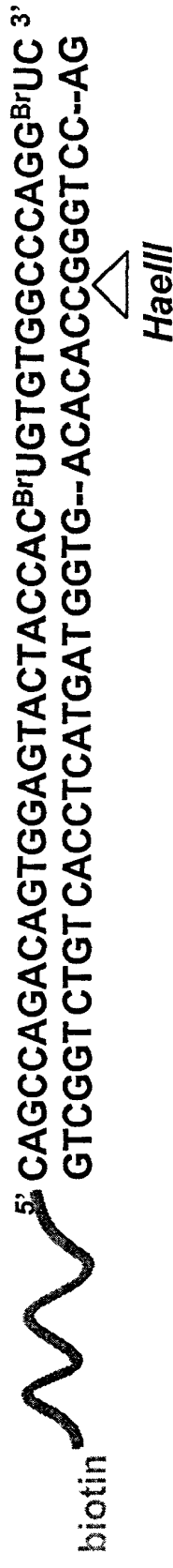


Figure 29

## Oligonucleotide Duplex Substrate for Mre11 Plate-Based Assay



Sequence was taken from oligonucleotide DG51 (Paul and Gellert, Mol. Cell, 1998), a substrate used to characterize the *in vitro* nuclease activity of recombinant Mre11. A HaeIII cleavage site was incorporated as a positive control for the assay.

Figure 30

# Biochemical Assay for Mre11 Exonuclease Activity

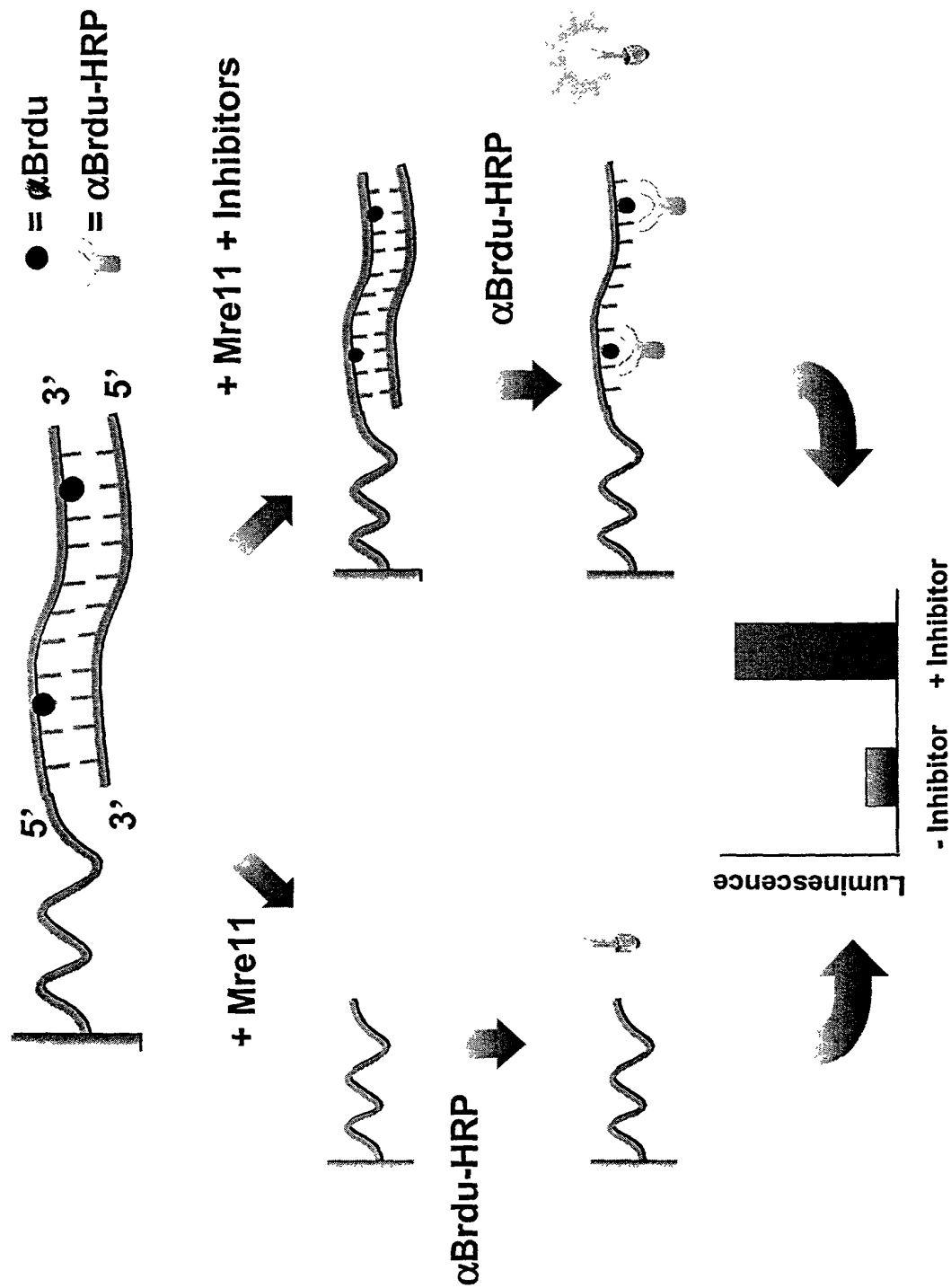


Figure 31

# Cleavage of Double-stranded Biotinylated Reporter by Mre11

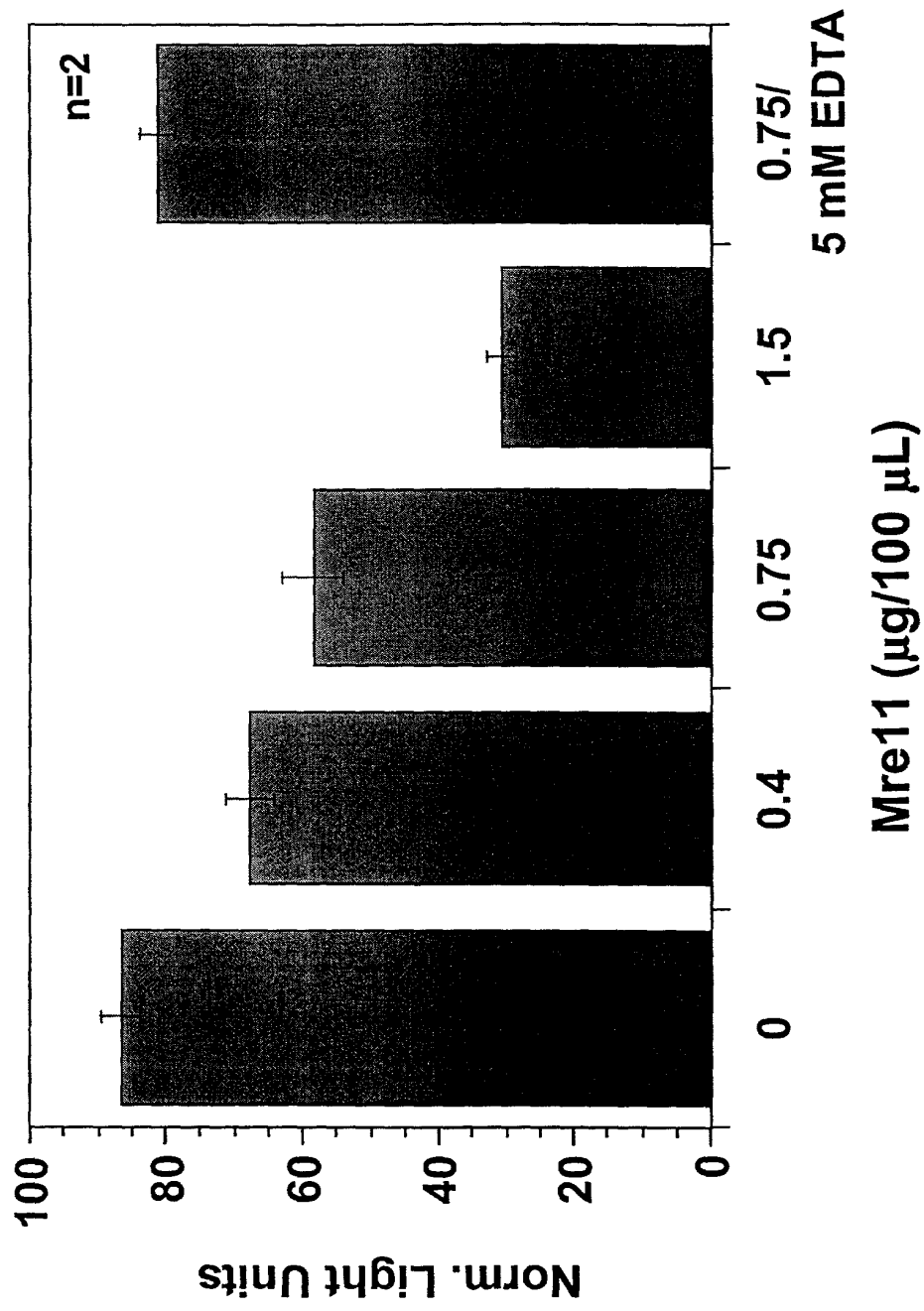
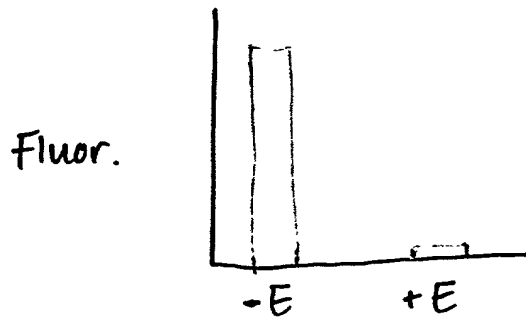
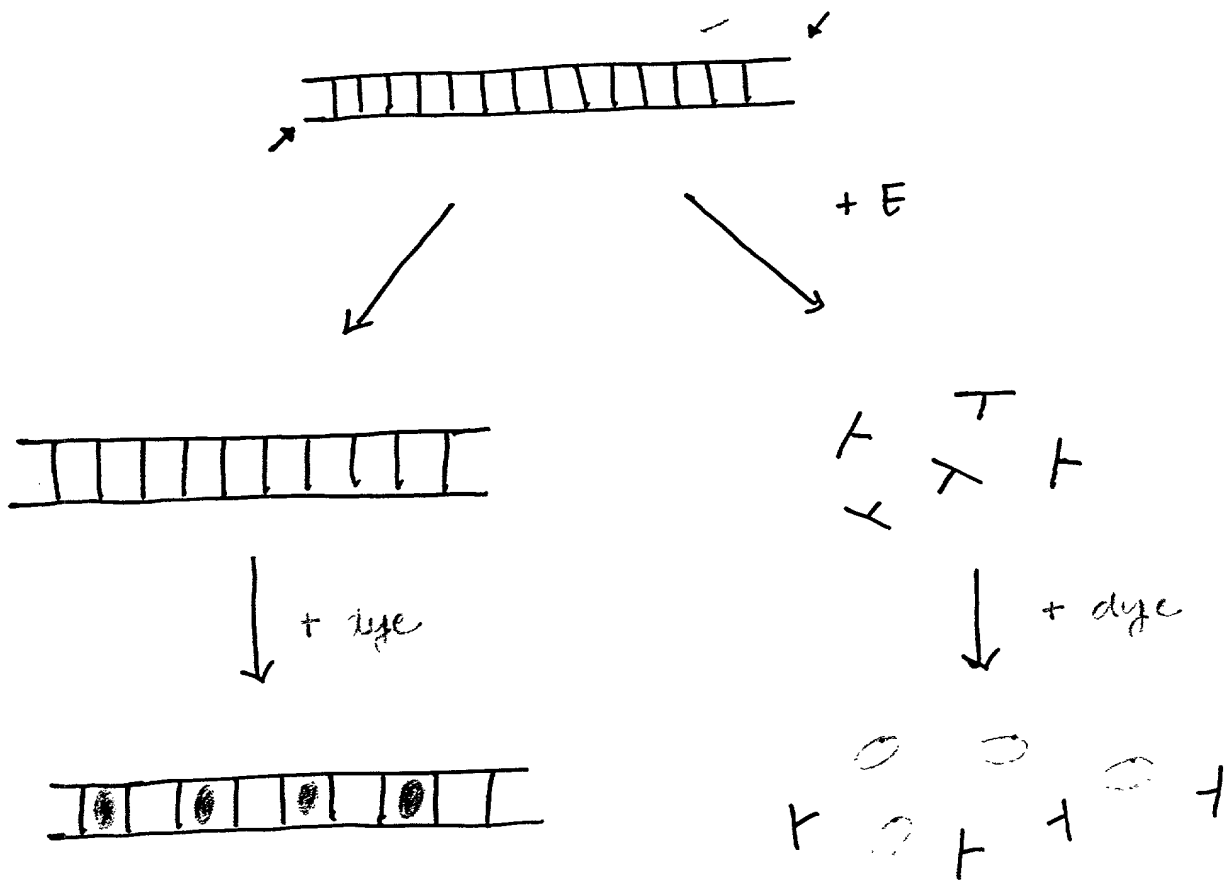


Figure 32

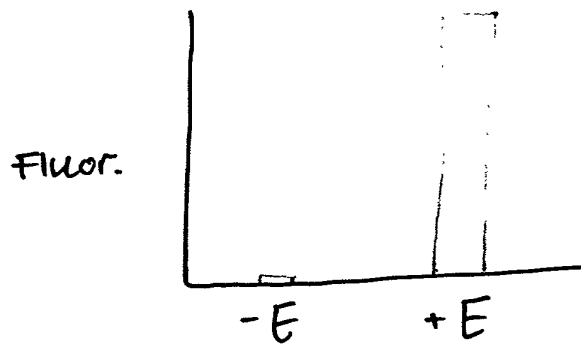
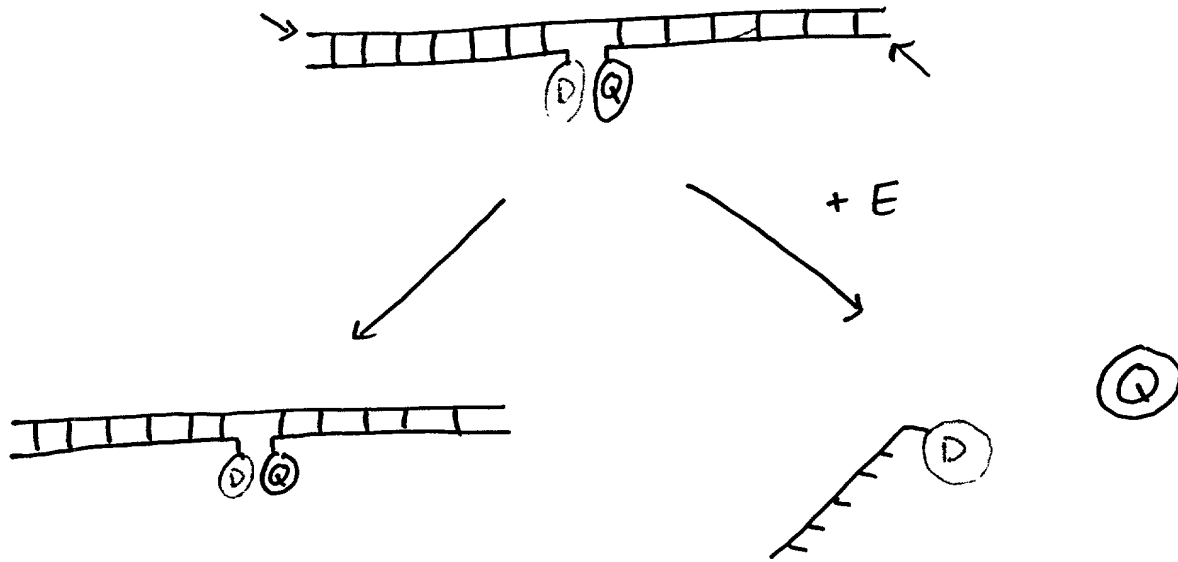
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Picogreen Dye Assay



Figure 33



Fluorescence Quenching Assays